PB81-212219

Chlorine Dioxide Water Disinfection
A Prospective Epidemiology Study

(U.S.) Health Effects Research Lab.
Cincinnati, OH

1981

U.S. Department of Commerce
National Technical Information Service
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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. RECIP
EPA-600/J-81-174	Journal Article	PB81-212219

4. TITLE AND SUBTITLE	5. REPORT DATE
Chlorine Dioxide Water Disinfection: A Prospective Epidemiology Study	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) G.E.Michael, R.K.Miday, J.P.Bercz, R.G.Miller, D.G. Greathouse, D.F.Kraemer, J.B.Lucas	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. EPA Health Effects Research Laboratory 26 W. St. Clair St. Cincinnati, Ohio 45268	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

Published: Archives of Environmental Health 36(1):20-27 (Jan/Feb 1981)

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA-600/J-81-174
Journal Article

Archives of Environmental Health is published by Heldref Publications, 4000 Albemarle St., N.W., Washington, D.C. 20016.

Chlorine Dioxide Water Disinfection: A Prospective Epidemiology Study

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ABSTRACT. An epidemiologic study of 198 persons exposed for 3 months to drinking water disinfected with chlorine dioxide was conducted in a rural village. A control population of 118 nonexposed persons was also studied. Pre-exposure hematologic and serum chemical parameters were compared with test results after 115 days of exposure. Chlorite ion levels in the water averaged approximately 5 ppm during the study period. Statistical analysis (ANOVA) of the data failed to identify any significant exposure-related effects. This study suggests that future evaluations of chlorine dioxide disinfection should be directed toward populations with potentially increased sensitivity to hemolytic agents.

RECENT RESEARCH STUDIES have demonstrated that chlorine (Cl_2) disinfection of drinking waters that contain naturally occurring humic substances and some synthetic organics results in the formation of trihalomethanes—principally chloroform. Since chloroform and some other halogenated compounds are demonstrated carcinogens, alternative water treatment chemicals and methods are being evaluated.[1-5]

Chlorine dioxide (ClO_2) is one of several alternate disinfectants being investigated by the Environmental Protection Agency which do not produce trihalomethane by-products. Chlorine dioxide also meets most requirements set forth by the Disinfectants Committee of the American Water Works Association as desirable, namely: (1) easy on-site generation with only minor modification of existing equipment; (2) excellent biocidal qualities; (3) reliable methodology for monitoring; and (4) relative cost-effectiveness.[6]

Chlorine dioxide is an oxidative disinfectant and chlorite ion (ClO_2^-) is rapidly formed as a major reduction product. Miltner[7] described some of the dynamics involved in ClO_2 water treatment and found 48% reduction to ClO_2^-, 22% conversion to chlorate (ClO_3^-), with 28% being further reduced to chloride (Cl^-) after 42 hr contact time. While both ClO_2^- and ClO_3^- are classified as methemoglobinemia-inducing agents, the greater concentration and oxidation potential of ClO_2^- make it the species of major concern.

Chlorite oxidizes hemoglobin to methemoglobin both in vitro and in vivo. Long-term studies in rats consuming water containing 100 mg/L had no apparent effects.[8] Musil et al.,[9] however, reported decreased hematocrit and hemoglobin after only 40 days in rats drinking surface water treated with ClO_2 having a residual of 0.2 mg/L. Recently Heffernan et al.[10] have identified a slight, but compensated anemia in rats exposed to 500 mg/L in drinking water for 90 days. Initial decreases in hemoglobin, red cell count, and packed cell volume observed after 30 days exposure had normalized by 90 days. Dose-related decreases in erythrocyte glutathione levels noted at ClO_2^- levels as low as 50 mg/L remained low after 90 days expo-

sure. Heffernan et al.[10] also demonstrated methemoglobin formation with single oral doses of sodium chlorite ($NaClO_2$) as low as 20 mg/kg body weight and hemolytic anemia in cats, evidenced by increased red cell turnover at doses of 3.0 mg/kg body weight and above. In vitro experiments with human red cells have demonstrated crenation, depletion of erythrocyte glutathione, stimulated erythrocyte hydrogen peroxide production, and methemoglobin formation from $NaClO_2$ concentrations of 10^{-3} to 10^{-1} *M*.[11] These data suggest that ClO_2^- catalyzes the formation of hydrogen peroxide (H_2O_2) within the cell, which is responsible for the decrease in glutathione concentrations, and that interference with glutathione perioxidase may be the critical effect. The literature on human ClO_2 and ClO_2^- toxicity, as related to exposure from use as a water disinfectant, is sparse, but has been the subject of recent review and speculation.[12,13] Enger[14] noted nausea in laboratory workers who drank 1 L of water containing 0.7 mg of ClO_2, but did not note any nausea in those drinking a concentration of 0.3 mg/L. Hoft[15] reported a "furry" sensation of the tongue in volunteers drinking a solution of 0.3 mg/L of $NaClO_2$. Soviet workers have investigated the organoleptic properties of ClO_2 in water.[16] A concentration of 0.40 to 0.45 mg/L was identified as the odor threshold. The Soviet group also noted that approximately 30% of the ClO_2 was "fixed" by the oral mucus membranes, and that 90% reaching the stomach was inactivated, which suggested a low probability of systemic effects. Other chlorinated species were not determined in their study, but the work of Miltner[7] suggests that at the pH of gastric juice, CLO_2 would be largely converted to ClO_2^- and ClO_3^-. No epidemiologic studies of communities served by utilities using ClO_2 for water disinfection have been previously reported.

Despite the paucity of data on the risks of ClO_2 disinfection to the general public, it has been recommended that ClO_2^- residuals should be nil, based on the possible threat to infants who have a decreased ability to reduce methemoglobin.[9,17] Based on Musil's report, the Norwegian Health Authority has recommended the total absence of residual ClO_2^- in drinking water.[18] Similarly, in Germany the applied dose of ClO_2 is unofficially limited to 0.3 mg/L to prevent possible adverse health effects.[9] Fridyland and Kagan[16] have disagreed with the recommendations of Musil et al., stressing that ClO_2 in water has low toxicity and may not be absorbed into the bloodstream, and suggested that any standard should be based on limiting organoleptic effects which occur at levels at least 10 times lower (0.40 to 0.45 mg/L) than concentrations causing adverse health effects.

Chlorine dioxide has received relatively limited use as a water disinfectant in the United States. An inventory of public water supplies has identified a small number of plants which use low levels of ClO_2 supplementally, as needed, to control periodic phenolic taste and odor problems.[20] Only one community was found which used ClO_2 exclusively for disinfection, and even that use was limited to a seasonal basis. It is a small Appalachian village with 250 homes plus a few stores in the hill lands of southeastern Ohio. Coal mining is the major industry of the region.

During the past 5 yr, the community used conventional Cl_2 treatment during the winter months, followed by total ClO_2 treatment from April to October. This annual changeover provided an opportunity to conduct a before-and-after comparison of selected health parameters in the consumers.

Chlorine dioxide was generated in this community's water system by feeding an aqueous solution of $NaClO_2$ and Cl_2 into a Wallace and Tiernan reactor. The chemical feed rate during an 8-hr production day varied with raw water conditions, and was entirely controlled by a small pump arrangement devised by the plant operator.

METHODS

Study population selection. Prior to ClO_2 treatment, individual volunteers were solicited from the village using ClO_2, and also from surrounding areas where ClO_2-treated water was not used. Written informed consent was obtained from each volunteer after the nature of the study and attendant risks of venipuncture were individually explained. A total of 351 persons donated blood samples and provided information concerning age, sex, water source, and medical history, including medication use, recent blood loss, acute and chronic illness, and personal water consumption. One hundred fifteen days after ClO_2 treatment was initiated for the study community, the survey was repeated and 327 of the original participants returned for resampling. Both before and after hematologic and serum chemistry results were available from 309 of the participants (197

Table 1.—Study Participants Classified by Age, Sex, and Chlorine Dioxide (ClO_2) Exposure

	Participants Drinking ClO_2-Treated Water			Participants Not Drinking ClO_2-Treated Water		
Age of Participants (yr)	Male	Female	Total	Male	Female	Total
<15	9	14	23	6	6	12
15 to 64	64	75	139	37	53	90
>64	14	21	35	5	5	10
Total	87	110	197	48	64	112

Table 2.—Comparability of the Titrimetric and Colorimetric Methods for the Analysis of the Chlorine Dioxide, Free Available Chlorine, and Chlorite

Sample Number	Date	Chlorine Dioxide		Free Available Chlorine		Chlorite	
		DPD Titri.*	DPD Color.†	DPD Titri.	DPD Color.	DPD Titri.	DPD Color.
1	4/24	.76	.21	.80	.46	5.04	5.26
2	4/25	.57	.29	.80	.31	6.65	6.10
3	4/26	.57	.76	.80	.22	5.32	4.94
4	4/27	.57	.49	.70	.21	4.61	4.01
5	5/01	1.14	<.05	.70	.32	3.13	3.76
6	5/02	.57	<.05	.65	.35	3.23	3.33
7	5/31	1.10	.25	.72	.39	5.80	5.45

NOTE: All values expressed as ppm.

*Titri = titrimetric test.

†Colorimetric test conducted 2 or more hr after titrimetric test.

ClO_2-exposed subjects and 112 individuals not exposed to ClO_2). The age and sex distributions for both participant groups are similar (Table 1).

Clinical pathology tests. Clinical pathology tests were limited to those which could be performed with minimum staff and equipment in the field, and which required only a single venipuncture per sampling period. Eleven tests were selected: hematocrit, hemoglobin, red cell count, white cell count, mean corpuscular volume, methemoglobin, blood urea nitrogen, serum creatinine, total bilirubin, reticulocyte count, and qualitative osmotic fragility screen. Glucose-6-phosphate dehydrogenase (G-6-PD) levels were determined on the initial sample to identify persons with genetic deficiencies of this enzyme. Mean corpuscular volume was calculated using hematocrit and red cell count data. A blood smear was also prepared for cell morphology. No attempt was made to control the time of day of collection or the fasting state of the individuals.

Heparinized samples were processed on the site for methemoglobin and osmotic fragility determination. Slides for differential and reticulocyte counts were also prepared at the site of blood collection. The remaining samples were sent to the laboratory and processing was initiated within 6 hr after collection.

Quality assurance in the clinical pathology testing was provided by calibrating the Coulter model ZB1-6 cell counter and Hemoglobinometer using Coulter 4C™ normal and abnormal cell controls and Coulter HB-10 and HB-20 Hemoglobinometer™ controls. Chemistry results were checked by calibrating the Centrifichem-400 system with Dade Monitrol I and Monitrol II™ assayed controls and Dade unassayed control Level I and Level II™ sera in every run. Both hematology and chemistry controls were taken from the same lot numbers during each sampling period.

Table 3.—Time Study of Chlorine Compounds in Finished Water

Chlorine Compounds	Sample A*					Sample B*			
	0 hr	2 hr	4 hr	6 hr	7 hr	0 hr	2 hr	4 hr	6 hr
Chlorine dioxide	2.01	1.18	1.03	<.05	<.05	.80	.65	<.05	<.05
Free available chlorine	N.D.†	N.D.	N.D.	N.D.	N.D.	1.02	.54	.18	.18
Chlorite	N.D.	N.D.	N.D.	N.D.	N.D.	2.85	2.57	3.37	3.28

NOTE: All values expressed as ppm.

*Samples A and B were analyzed at different times of day.

†N.D. = Not determined.

Table 4.—Average Concentration of Chlorine Dioxide and Related Products by Week

Compound	Pre-ClO_2	Week 1	2	3	4	5	6	7	8	9	10	11	12	Post-ClO_2 13	14
Chlorine dioxide	<0.05	0.98	1.03	0.80	0.72	0.76	1.11	0.64	0.93	0.33	0.57	0.25	0.61	<0.05	<0.05
Free available chlorine	1.45	N.D.*	0.63	0.74	0.62	0.89	0.86	0.59	0.82	0.45	0.71	0.91	0.69	0.63	0.20
Chlorite	0.32	N.D.	6.96	5.99	3.92	5.24	6.64	5.41	5.57	6.17	3.65	3.19	3.68	1.40	0.50
Chlorate	<0.10	N.D.	1.13	1.13	0.62	0.58	0.84	0.66	1.04	0.89	0.63	0.34	0.34	0.10	0.15
Combined chlorine (chloramines)	0.38	N.D.	1.82	1.15	0.87	1.04	1.20	0.90	1.30	1.22	1.08	0.96	1.04	0.90	0.29
Total available chlorine	2.86	N.D.	20.83	16.96	11.87	15.54	19.10	14.83	16.95	15.89	10.07	8.79	11.14	4.55	1.61

NOTE: All values expressed as ppm.

*Not determined.

Drinking water analyses. Because of the very complex chemical interactions among anions of the oxo acids of chlorine, it was essential to obtain analytically proficient data on all chlorine compounds in the finished water. This study presented an opportunity to extensively compare different analytic methods for determining ClO_2. Initially, three different methods of ClO_2 analysis were used to generate data for comparison: (1) DPD-Colorimetric,[21] DPD-FAS-Titrimetric,[21] and (3) Amperometric.[22] During the first weeks of testing it became evident that the Amperometric method was not suitable because negative findings for combined clorine (monochloramines and dichloramines) prevented the calculation of other chlorine species (ClO_2) by difference. The two remaining methods were used to analyze for free available Cl_2, ClO_2^-, and ClO_2. Additional methods were used for the analysis of total available chlorine, monochloramine, dichloramine,[21] chlorate,[7] and pH of the raw and finished water.

Routine water samples were taken at the point where the water left the clear well. The DPD-Colorimetric analysis for free available Cl_2, ClO_2^-, and ClO_2, the ClO_3^- analysis, and the pH determinations were performed 5 days/wk, 2 hr or more after sample collection. All other analyses were performed by the DPD-Titrimetric method for 7 days/wk immediately following sample collection. Weekly samples from peripheral points in the water distribution system were also collected for control purposes. Two water samples were taken from the distribution system and analyzed for trihalomethane content using the Bellar purge and trap method.[23] The first sample was collected during the last week of ClO_2 disinfection, and the second sample was collected immediately following a return to conventional chlorine treatment.

Statistical analysis. The participants were classified by age (< 15 yr, 15-64 yr, > 64 yr), sex, and ClO_2 exposure (residence served or not served by ClO_2-treated water). Effects of ClO_2 exposure were assessed by examination of individual differences between clinical pathology test results for the blood samples collected before and after initiation of ClO_2 treatment in the test community. Differences in the chemical and hematologic parameters from March to June were compared with similar measurements in the unexposed population. Each person served as his/her own control. In order to test for the effects of age and sex and their interaction with ClO_2 exposure on the individual differences, a three-way analysis of variance (ANOVA) was used for the primary statistical analysis; an overall significance level of $P < .05$ was selected.

To determine if a particular factor classification(s) (e.g., < 15 yr, 15-64 yr, or > 64 yr) was significant for a significant factor (e.g., age), the Duncans Multiple Range Test[24] was used with $P < .05$. The underlying statistical assumptions of ANOVA were tested and appropriate mathematical transformation[8] (e.g., square root) was applied where needed.

The ANOVA was performed using the available computerized statistical package [Statistical Analysis System, revision 76.5, procedure GLM (general linear models)].[25]

RESULTS

Water analysis. Data comparisons for ClO_2 and free available Cl_2 using titrimetric and colorimetric methods revealed substantially lower values with the latter technique (Table 2). This led to a time study, since there was a lag of 2 hr or more between the performance of the two methods. Table 3 demonstrates the effect of time. Methods were compared when there was a minimum (20 min) sample storage. Both ClO_2 and free available Cl_2 rapidly disappear from water after initial formation. Clorine dioxide decay also occurs irrespective of dark vs. light sample storage. Therefore, no comparison of analytic methodology

Table 5.—Twenty-Four-Hour Study of Chlorine Compounds

Chlorine Compounds	0* hr	2 hr	4 hr	6 hr	8† hr	10 hr	12 hr	14 hr	16 hr	18 hr	20 hr	22 hr	24* hr	31 hr
Chlorine dioxide	.49	.80	1.33	1.60	2.01	.65	.49	<.05	<.05	<.05	<.05	<.05	<.05	.68
Free available chlorine	.74	1.02	1.10	.94	.86	.34	.06	.36	.24	<.01	<.01	<.01	.56	1.20
Monochloramine as chlorine	.50	.48	.48	.72	.60	1.00	.56	.40	.44	.48	.52	.24	.56	.44
Dichloramine as chlorine	.40	.60	.52	.48	.40	.20	.16	.64	.56	.56	.68	.80	.44	.52
Chlorite	5.56	2.85	2.19	2.60	1.19	2.02	2.96	2.93	3.31	3.34	3.06	3.23	3.27	2.87
Total available chlorine §	15.30	10.68	10.44	10.50	9.88	7.68	8.80	7.84	8.28	8.08	7.64	7.60	8.48	10.48

NOTE: All values expressed as ppm.

*ClO_2 generation started.

†ClO_2 generation stopped.

§Sum of all analyzed species expressed as chlorine.

Table 6.—Summary of Laboratory Test Results for a Population Exposed to Chlorine Dioxide-Treated Drinking Water and a Comparison Unexposed Population

Test	Normal Values	ClO_2-Exposed Population		Population Not Exposed to ClO_2		Difference between Means	
	Range	March*‡	June†‡	March*‡	June†‡	Exposed June-March	Unexposed June-March
Hematocrit (%)							
male	42-52	45.13 ± 3.68	44.45 ± 3.82	44.92 ± 3.61	44.08 ± 3.23	−0.68	−0.84
female	37-47	41.03 ± 3.46	40.46 ± 2.97	40.67 ± 3.32	39.50 ± 3.26	−0.59	−1.17
Hemoglobin (g/dl)							
male	14-18	15.02 ± 1.28	14.80 ± 1.32	15.04 ± 1.32	14.86 ± 1.16	−0.22	−0.18
female	12-16	13.53 ± 1.18	13.28 ± 1.05	13.34 ± 1.13	13.15 ± 1.19	−0.25	−0.19
Red cell count ($10^6/mm^3$)							
male	4.4-6.0	5.01 ± 0.37	4.95 ± 0.40	5.06 ± 0.40	4.98 ± 0.42	−0.06	−0.08
female	4.2-5.5	4.58 ± 0.36	4.51 ± 0.34	4.58 ± 0.35	4.51 ± 0.36	−0.07	−0.07
White cell count ($10^3/mm^3$)							
male	4.3-10.0	7.65 ± 1.96	7.00 ± 1.65	7.38 ± 1.64	7.16 ± 1.73	−0.65	−0.22
female	4.3-10.0	7.49 ± 1.88	6.92 ± 2.02	7.71 ± 1.81	7.15 ± 1.89	−0.57	−0.56
Reticulocyte count (%)							
male	0.8-2.5	0.90 ± 0.49	0.80 ± 0.46	0.86 ± 0.37	0.75 ± 0.39	−0.10	−0.11
female	0.8-4.1	0.82 ± 0.40	0.78 ± 0.41	0.95 ± 0.42	0.76 ± 0.41	−0.04	−0.19
Mean corpuscular volume (u^3)							
male	82-101	90.47 ± 3.81	90.39 ± 4.06	89.25 ± 4.18	88.88 ± 3.88	0.08	−0.37
female	82-101	90.24 ± 4.34	90.23 ± 4.31	89.06 ± 5.03	88.55 ± 5.03	−0.01	−0.51
Methemoglobin (%)							
male	0-1.6	0.09 ± 0.05	0.09 ± 0.06	0.08 ± 0.04	0.11 ± 0.05	0.00	0.03
female	0-1.6	0.09 ± 0.06	0.06 ± 0.05	0.09 ± 0.05	0.07 ± 0.05	−0.03	−0.02
Blood urea nitrogen (mg/dl)							
male	5-26	16.22 ± 4.96	15.91 ± 4.78	15.89 ± 3.73	16.60 ± 4.08	−0.31	−0.71
female	5-26	15.04 ± 4.75	14.77 ± 4.93	14.16 ± 4.53	15.14 ± 4.38	−0.27	0.98
Serum creatinine (mg/dl)							
male	0.5-1.3	1.18 ± 0.24	1.15 ± 0.24	1.16 ± 0.24	1.17 ± 0.22	−0.03	0.01
female	0.5-1.3	1.00 ± 0.20	0.99 ± 0.18	0.99 ± 0.19	0.99 ± 0.13	−0.01	0.00
Total bilirubin (mg/dl)							
male	0-1.5	0.78 ± 0.41	0.94 ± 0.52	0.77 ± 0.46	0.88 ± 0.37	0.16	0.11
female	0-1.5	0.60 ± 0.33	0.70 ± 0.31	0.56 ± 0.37	0.64 ± 0.27	0.10	0.08

*One week before the test community initiated chlorine dioxide treatment of their drinking water.

†Ten weeks after the test community initiated chlorine dioxide treatment of their drinking water.

‡Values expressed as mean ± standard deviation.

was possible, except for the analysis of ClO_2^- (Table 2) where the results, considering the analytic parameters and method of calculation by difference from total other chlorine species, were in good agreement. Therefore, all data calculations were based upon the titrimetric method. All data are expressed as parts per million (ppm) of the species, except the mono- and dichloroamines, which were calculated as Cl_2.

A summary of the weekly mean concentration of ClO_2 and related compounds is reported in Table 4. The greatest week-to-week variability was seen with the ClO_2^- concentration which ranged from approximately 3 to 7 ppm as a weekly average through the 12-wk exposure period. The daily mean ClO_2^- level was 5.21, based on 70 duplicate analyses, with a range of 2.99 to 8.55 ppm. Chlorate levels obtained from 42 samples ranged from 0.17 to 1.79 ppm with a mean of 0.73 ppm. All other Cl_2 compounds were relatively consistent when observed on a weekly average basis. The pH values of the raw water ranged from 7.8 to 8.4, with a slight rise being noted as the reservoir warmed with the onset of summer. The pH of the finished water ranged between 9.1 to 9.8, but without a trend over time.

The water plant was operated only 8 hr/day and the community was served by elevated storage the rest of the day. A 24-hr study was undertaken to examine daily variations in the concentration of the Cl_2 species (Table 5). Chlorine dioxide and free available Cl_2 disappeared within 2 to 4 hr after cessation of ClO_2 generation as previously noted (Table 2). Chlorite concentrations increase with the disappearance of ClO_2.

Chlorite levels for samples collected from various points along the distributor system ranged from 2.42-7.03 ppm, with a mean concentration of 5.28 (14 measurements).

Trihalomethane compounds were found to total 16 ppm in the distribution sample collected during ClO_2 treatment and 284 ppm following a return to conventional chlorination. Chloroform was the preponderate trihalomethane (≥ 95%) in both samples.

To assess individual exposures to ClO_2 by-products, water consumption information was obtained from each participant at the second sampling. The amount of water consumed—1.98 ± 1.10 L/day [mean ± standard deviation (*SD*)]—was used to calculate the range and mean chlorite dose received: 0-39.4 mg/day and 10.3 mg/day, respectively.

Hematologic serum chemistry results. A summary of the March and June group mean values for each of the 10 hematologic and serum chemical parameters measured is presented in Table 6. The group mean differences in exposed and control populations are generally similar in magnitude and direction of change. The amount of change from March to June in all parameters is small in relation to normal physiologic variability and laboratory measurement precision. There was a slight decrease between samplings in hematologic indices in both the exposed and control groups, which might have resulted from a seasonal effect or from laboratory variation.

Results of the analysis of variance performed on the March to June difference values are presented in Table 7. For each blood parameter, *P* values are listed for the three individual factors tested (i.e., exposure status, age category, and sex) and their interactions. Four single parameters—blood urea nitrogen and exposure, methemoglobin and sex, hemoglobin and age, bilirubin and age—and one three-way interaction—red cell count and exposure•sex•age—achieved statistical significance ($P < .05$).

The change in methemoglobin in the males vs. females was the only highly significant effect ($P < .0004$). This resulted from a slight increase in the male values while the female values decreased. These changes were independent of exposure category or age class.

The blood urea nitrogen was the only factor that showed a significant relationship to exposure. The direction of change, however, was opposite of that expected to result from toxic renal effects of ClO_2. The exposed group decreased 0.28 mg/dl, while the unexposed group increased 0.86 mg/dl. There was no consistent relationship to age class or sex.

Bilirubin changes were significantly different among the three age classes. This resulted from a greater increase in the youngest age class (0.28 mg/dl), compared with slight increases in the middle and older age groups (0.10 and 0.07 mg/dl). There was no sex or exposure association. Although neither the group nor individual values of the children were elevated above the normal range, there was a 51% increase in total bilirubin level in this age group from March to June.

A slight increase (0.04 g/dl) in hemoglobin values in this age group was also statistically significant ($P = < .045$), compared with decreases in the other two age groups (0.23 and 0.40 g/dl). This effect was caused by increased hemoglobin values in young female subjects (0.20 g/dl), while all other age-sex classes showed decreases of similar magnitude. The hematocrit and red blood cell differences followed the same pattern, with *P* values of .077 and .059.

DISCUSSION

This prospective cohort epidemiologic study failed to identify any adverse effects associated with a 12-wk exposure to ClO_2-disinfected drinking water. Although no differences were noted after 12 wk, we cannot rule out transient effects occurring during the exposure period. Mild transient hemolytic anemias that recovered during continuing exposure were identified by Heffernan[10] in rats exposed to ClO_2^-. However, any hematologic toxicity of clinical significance in this population should have been manifest with some persistent findings at the 3-month time point.

A more critical issue may be the presence of sensitive individuals in the population tested. The present study was limited by a lack of racial and ethnic diversity among the village population. Only one volunteer manifested a glucose-6-phosphate deficiency. It is worth noting that this individual's red cell count, hemoglobin, and hematocrit declined during the exposure period and subsequently increased 3 months following cessation of exposure (Table 9). There was no noteworthy change in methemoglobin content or other parameters. The subject was an adult onset diabetic, who controlled the condition with oral hypoglycemic agents. Whether the decline in hematologic parameters seen in this person represents a random event, or is causally related, is unknown. Nonetheless,

Table 7.—Statistical Significance of Chlorine Dioxide Exposures, Age, Sex, and Their Interactions and ANOVA Results for March to June Test Differences*

Test	Exposure	Sex	Age	Exposure† Sex	Exposure† Age	Sex† Age	Exposure* Sex* Age
Hematocrit (%)	.212	.786	.877	.470	.385	.109	.174
Hemoglobin (g/dl)	.561	.782	.045†	.941	.860	.245	.088
Red cell count (10^6/mm^3)	.764	.907	.059	.893	.666	.329	.023†
White cell count (10^3/mm^3)	.260	.643	.080	.360	.522	.258	.206
Reticulocyte count (%)	.119	.921	.469	.253	.911	.370	.590
Mean corpuscular volume (u^3)	.155	.968	.541	.578	.455	.539	.565
Methemoglobin (%)	.091	.0004†	.621	.185	.608	.662	.106
Blood urea nitrogen (mg/dl)	.024†	.743	.112	.896	.453	.654	.365
Serum cretainine (mg/dl)‡	.622	.340	.511	.734	.361	.313	.725
Total bilirubin (mg/dl)§	.716	.807	.012†	.771	.356	.239	.694

NOTE: All numbers represent *P* values.

*Individual differences were computed for each participant by subtracting the clinical pathology tests results for the time period prior to chlorine dioxide use from the results of the repeat samples 10 wk after initiation of chlorine dioxide treatment.

†$P < .05$

‡A square root transformation was used to normalize the residuals of the three-way analysis of variance.

§Raw and transformed data failed to meet normality assumptions. All analyses, including non-parametric ANOVA, had non-significant results.

G-6-PD-deficient individuals represent a group of special concern for oxidant 2n-induced hematotoxicity as suggested by Moore et al.,[12] and represent a priority area for further study in the evaluation of ClO_2. Infants are also a high-risk group that were not included in the present study. Their susceptibility to oxidant-induced methemoglobinemia is well documented.

Several statistically significant associations were found that were not related to our primary hypothesis; all involve minor shifts in blood parameters within the normal range. Although none of these changes indicate pathologic effects, several may be real physiologic changes associated with seasonal influences or other unknown variables. The strongest association found was for methemoglobin and sex. Blood levels in male and female participants changed in opposite directions from March to June—male values increasing while female values decreased. There is no obvious explanation why a seasonal influence would affect the two sexes differently. Different dietary habits or levels of physical activity in the two groups might have some influence. Because the effect is consistent across age and exposure categories, and because systematic laboratory variation could not explain the finding, it appears to be a real, though slight, physiologic response.

Both total bilirubin and hemoglobin concentration were slightly elevated in the youngest age group in comparison with the two older age classes. These may be compensatory changes related to increased physical activity and sensitivity of erythrocytes to thermal stress in the children.

The only significant change found related to exposure was increased blood urea nitrogen levels in the unexposed group. If this is a real difference, the most likely explanation is that this group experienced mild dehydration in June. The second sampling was conducted during extremely hot weather and a greater proportion of the unexposed control group was employed actively outdoors. The blood urea nitrogen/creatinine ratio was examined and the pattern was similar to the blood urea nitrogen changes, with greater increases in the unexposed subjects, supporting a relative volume depletion explanation.

Table 8.—Hematologic Parameters in a Glucose-6-Phosphatase Deficient Individual

Test	Pre-Exposure	Immediate Post-Exposure	90 Days Post-Exposure
Red cell count (10^6/mm^3)	4.78	4.11	4.43
Hemoglobin (g/dl)	14.70	12.90	14.00
Hematocrit (%)	46.0	40.0	42.0

Although there was no evidence of pathologic disturbances in the community consuming ClO_2-disinfected drinking water, the question of dose-response effects related to individual exposures was not specifically addressed. Because drinking water intake was highly variable, effects that occurred only in the highest ClO_2-dose group might have been masked by analyzing all exposed subjects as one group. We examined this possibility by stratifying results for each test parameter by water intake, within the exposed group. No dose-response trends were noted.

In summary, we found no association between the use of ClO_2-treated water and adverse health effects, as evidenced by multiple hematologic and serum clinical chemistry measurements. Several serum parameter changes associated with specific age and sex categories that were statistically significant were also small in magnitude and were not considered to be of pathologic importance. The results of this study suggest that future research of ClO_2 disinfection should be focused on "high-risk" individuals susceptible to oxidant stress.

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The authors gratefully acknowledge the technical and field assistance provided by: Helen Ball, William J. Benton, John Bisbocci, Lucille Garner, Emma Lou George, Herbert Muller, Lillian L. Jones, Charles J. Mayhew, Janice H. Minar, Donna T. Murray, Bradford L. Smith, Verna E. Tilford, and Steven C. Waltrip. Special thanks is due Debbie Brock and Debbie Dean for their help with the manuscript.

Submitted for publication October 15, 1979; revised; accepted for publication December 5, 1980.

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REFERENCES

1. Rook, J. J. 1974. Formation of haloforms during chlorination of natural waters. *Water Treatment and Examination*. 23: 234-43.
2. Bellar, T. A.; Lichtenberg, J. J.; and Kroner, R. C. 1974. The occurrence of organohalides in chlorinated drinking water. *J Am Water Works Assoc* 66: 703-06.
3. Symons, J. M.; Bellar, T. A.; Carswell, J. K.; DeMarco, J.; Kropp, K. L.; and Stevens, A. A. 1975. National organics reconnaissance survey for halogenated organics in drinking water. *J Am Water Works Assoc* 67: 634-47.
4. Rook, J. J. 1976. Haloforms in drinking water. *J Am Water Works Assoc* 68: 168-72.
5. National Cancer Institute. 1976. *Report on the Carcinogenesis Bioassay of Chloroform*. Springfield, VA: National Technical Information Service PB-264018.
6. American Water Works Association Committee on Disinfection. 1978. *J Am Water Works Assoc* 70: 219-22.
7. Miltner, R. J. 1977. Measurement of chlorine dioxide and related products. *Proceedings AWWA Water Quality Technology Conference*, December 6-7, 1976, Paper No. 2A-5. San Diego, California.
8. Haller, J. F.; and Northgraves, W. W. 1955. Cited in *Berg Tappi* 38: 199.
9. Musil, J.; Kontek, Z.; Chalupa, J.; and Schmidt, P. 1963. Toxicological aspects of chlorine dioxide application for the treatment of water containing phenol. *Sf uvy sk. Chem-Technol. Praze* 8: 327-345.
10. Heffernan, W. P.; Guion, C.; and Bull, R. J. 1979. Oxidative damage to the erythrocyte induced by sodium chlorite, in vivo. *J Environ Pathol Toxicol* 2: 1487-1496.
11. Heffernan, W. P.; Guion, C.; and Bull, R. J. 1979. Oxidative damage to the erythrocyte induced by sodium chlorite, in vitro. *J Environ Pathol Toxicol* 2: 1501-1510.
12. Calabrese, E. J.; Moore, G. S.; and Tuthill, R. W. 1978. The health effects of chlorine dioxide as a disinfectant on potable water: A literature survey. *J Environ Health* 41: 26-31.
13. Moore, G. S.; Calabrese, E. J.; DiNordi, S. R.; and Tuthill, R. W. 1978. Potential health effects of chlorine dioxide as a disinfectant in potable water supplies. *Med Hypotheses* 4: 481-496.
14. Enger, M. 1960. Treatment of water with chlorine dioxide to improve taste. *Gas and Wasser Fach* 14: 340-43.
15. Hoft, W. 1967. Experiments with chlorine dioxide treatment of drinking water. *Gas and Wasser Fach* 30: 852-54.
16. Fridlyand, S. A.; and Kagan, G. Z. 1971. Experimental validation of standards for residual chlorine dioxide in drinking water. *Hyg Sanitation* 36: 18-21.
17. Berndt, H. 1965. Studies on water treatment and disinfection with chlorine dioxide, particularly on the problem of pH-value dependence and chlorine residual. *Arch Hyg Bacteriol* 148: 10-43.
18. Myhrstad, J. A.; and Samdal, I. E. 1969. Behavior and determination of chlorine dioxide. *J Am Water Works Assoc* 61: 205-08.
19. Van Haren, F. W. J. 1970. Processes for the removal of organic pollutants. In *Water Treatment in the 70s*, pp. 113-128. University of Reading, England: Joint Society of Water Treatment Engineer/Water Research Association Symposium (January 5-7).
20. Inventory of Public Water Supplies. 1977. Office of Drinking Water, Washington, D.C.: U.S. Environmental Protection Agency.
21. Palin, A. T. 1974. Analytical control of water disinfection with special reference to differential DPD methods for chlorine, chlorine dioxide, bromine, iodine and ozone. *J Inst Water Engr* 28: 139-54.
22. American Public Health Association. 1975. Chlorine dioxide/amperometric method, Part 411C. In *Standard Methods for the Examination of Water and Wastewater*, 14th Ed., M. C. Rand, A. E. Greenberg, and M. J. Taras, eds., pp. 357-58. Washington, D.C.: American Public Health Association.
23. United States Environmental Protection Agency. 1979. *The Analysis of Trihalomethanes in Finished Waters by the Purge and Trap Method* (Method 501.1). Cincinnati, OH: Environmental Monitoring and Support Laboratory, United States Environmental Protection Agency.
24. Sokal, R. R.; and Rohlf, F. J. 1969. Biometry. *The Principles and Practice of Statistics in Biological Research*. San Francisco, California: W. H. Freeman and Company.
25. Barr, A. J.; Goodnight, J. H.; Sall, J. P.; and Helwig, J. T. 1976. *A Users Guide to SAS*, pp. 127-44. Raleigh, N.C.: SAS Institute, Inc.
26. Helwig, J. T. (ed.). 1977. *SAS-Supplemental Library User's Guide*. Raleigh, N.C.: SAS Institute.